

# New Plant Introductions with Resistance to the Soybean Aphid

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## ABSTRACT

The soybean aphid (SA; *Aphis glycines* Matsumura) was first found in the northern soybean [*Glycine max* (L.) Merr.] growing regions of the United States in 2000. By 2005, the aphids had spread to 23 soybean growing states reaching as far south as Mississippi and Georgia and also north into Ontario, Canada. The objective of this study was to identify new sources of resistance to the SA. Nearly 200 soybean genotypes (cultivars, breeding lines, and plant introductions [PIs]) were screened for resistance to SA in a greenhouse choice test using SA collected in Wooster, OH. Three PIs (PI 243540, PI 567301B, and PI 567324) were identified as resistant while six PIs were identified as moderately resistant. The findings on the three resistant and three of the six moderately resistant PIs were confirmed through further field and greenhouse tests. PI 243540 displayed strong antibiosis resistance such that SA was unable to survive on this PI in a no-choice test. The other two resistant PIs possessed mainly antixenosis type resistance. PI 243540 and PI 567301B were also resistant to the SA collection from Illinois. The aphid resistant PIs identified in this study will be useful in efforts to develop aphid-resistant soybean cultivars.

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**Abbreviations:** ASI, aphid susceptibility index; DAI, days after infestation; LSD, least significant difference; MG, maturity group; OARDC, Ohio Agricultural Research and Development Center; PI, Plant Introduction; RCBD, randomized complete block design; SA, soybean aphid.

THE SOYBEAN APHID (SA; *Aphis glycines* Matsumura) was first found in the northern soybean [*Glycine max* (L.) Merr.] growing regions of the United States in 2000 (Hartman et al., 2001). The SA rapidly spread throughout the midwestern region and to other parts of the United States and also into Ontario, Canada (OMAFRA, 2002). By 2004, 80% of the U.S. soybean fields was infested by SA (Venette and Ragsdale, 2004). In 2005, the aphids had spread to 23 soybean growing states reaching as far south as Mississippi and Georgia. Many soybean fields in the North Central soybean growing states, including those in northern half of Ohio, crossed the economic threshold of infestation. Millions of dollars were spent for spraying chemicals to control the aphids in infested soybean fields (Li et al., 2007). Currently, the SA appears to be on a 2-yr cycle, a year with significant economic problems (2001, 2003, and

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2005) followed by a year where overall populations are low (2002, 2004, and 2006) (Ragsdale, 2006).

The SA is native to southeastern and eastern Asia and it has long been a pest of soybean in many Asian countries, including China, Japan, South Korea, the Philippines, Indonesia, Malaysia, Thailand, Vietnam, and Russia (Wu et al., 2004). In addition to the United States, the SA was also found in Canada and Australia in recent years (OMAFRA, 2002; Fletcher and Desborough, 2002). Among all insect pests of soybean in China, the SA is considered as the number one threat to soybean productivity (Sun et al., 2000). The SA is small and light yellow or yellowish green in color with two distinct black cornicles. A combination of the body color, black cornicles, and its colonization on soybean and buckthorn (*Rhamnus cathartica* L.) distinguishes it from other aphid species (Voegtlin et al., 2004).

Severe aphid infestations can result in a variety of visible symptoms that include curling, wilting, yellowing, and premature dropping of leaves (DiFonzo and Hines, 2002). Other phenotypic manifestation of SA feeding damage may include stunted plants, reduction in the number of pods and seeds, and reduced seed weight and seed quality (DiFonzo and Hines, 2002). Under certain conditions, significant yield loss can occur due to feeding damages from SA. In 2001, yield losses in Minnesota were greater than 50% with some plants having several thousand aphids (Ostlie, 2002). In China, the soybean yield was reduced up to 52% with an average of about 220 aphids per plant (Wang et al., 1994). Soybean aphids not only result in reduction of seed yield, but they also reduce seed quality (e.g., discoloration, deformation) which is a major concern for food-grade soybean growers and consumers.

In addition to the yield loss of soybean from the direct feeding damage, a potentially large threat posed by the aphid is its ability to transmit certain plant viruses such as *Alfalfa mosaic virus*, *Soybean dwarf virus*, and *Soybean mosaic virus* to soybean (Sama et al., 1974; Iwaki et al., 1980; Hartman et al., 2001; Hill et al., 2001). The SA have been reported to create serious problems in cucumber (*Cucumis sativus* L.), squash (*Cucurbita* spp.), pumpkin (*Cucurbita* spp.), and dry beans (*Phaseolus* spp.) by colonizing and transmitting viruses to these plants in Michigan (C. Di Fonzo, Michigan State University, personal communication, 2006). One big concern in the eastern Great Lakes region is the potential for the SA to increase transmission of viruses from nearby forage legumes to soybeans and visa versa. The SA is the first soybean-colonizing aphid in the United States, and the full extent of its consequences on future virus disease problems in soybeans and other crops is still unknown.

If the SA population is increasing and plants are in the late vegetative or early reproductive stages (R1–R4) (Fehr and Caviness, 1977), an economic threshold of 250 aphids per plant should be used for applying chemicals for

controlling SA to avoid economic damage (yield loss that exceeds the cost of control) to the soybean crop (Rice, 2004). However, scouting soybean fields to decide when to apply chemical control requires frequent scouting by experienced people, and aphid populations are strongly influenced by weather. Also, spraying soybean fields with insecticides to control aphids is costly, can kill beneficial insects, and may cause environmental pollution (Sun et al., 2000). Chemical control of SA is also unacceptable to the producers and consumers of organic soybean products.

One way of reducing the reliance on chemical control of SA is to grow soybean cultivars with aphid resistance. Because SA is a new pest of soybean in the United States, no aphid resistant cultivar is commercially available yet. The first step in developing resistant cultivars is to identify the genetic sources of resistance. Genetic resistance to SA in soybean germplasm has been reported in China (Fan, 1988; Sun et al., 1991). The resistance to SA also has been found in *Glycine soja* Sieb. and Zucc. (Yue et al., 1988, 1989). After the establishment of SA in North America, several research groups started to search for aphid resistant germplasm. Until now, three research groups have reported the identification of SA resistant germplasm (Hill et al., 2004; Mensah et al., 2005; Diaz-Montano et al., 2006). Hill et al. (2004) reported three soybean lines as resistant to SA. They reported two soybean genotypes—Dowling, a maturity group (MG) VIII cultivar, and Jackson, a MG VII cultivar—with antibiosis resistance (feeding on the plant results in mortality or disruption of growth, development, or physiology of the insect), and one plant introduction, PI 71506, with antixenosis or nonpreference resistance (the insect is either repelled from or not attracted to the host plant) against SA. Mensah et al. (2005) found four MG III plant introductions from Shandong province of China with aphid resistance; PI 567543C and PI 567597C have antixenosis resistance while PI 567541B and PI 567598B have antibiosis resistance. Diaz-Montano et al. (2006) reported 11 soybean genotypes with resistance to SA.

Aphid resistance in some plants has been found to be controlled by single gene or few genes (Klingler et al., 2005). The aphid resistance in each of the two soybean cultivars Dowling and Jackson is controlled by single dominant genes (Hill et al., 2006). The aphid resistance in the germplasm identified by Mensah et al. (2005) is also controlled by major genes (D. Wang, Michigan State University, personal communication, 2006). If a single gene for antibiosis resistance (such as the source from the Univ. of Illinois at Urbana, IL) is widely deployed, it is likely that the SA will overcome this resistance gene in a relatively short time. Therefore, pyramiding of multiple resistance genes, particularly genes with different modes of action, in the same cultivar has the potential of providing a higher level of SA resistance and/or a more durable resistance. The objective of this study was to identify additional soybean germplasm with resistance to SA.

## MATERIALS AND METHODS

### The Source of Aphids

A colony of SA was established in a growth chamber at Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, during the summer of 2005 by collecting aphids from nearby soybean fields. The colony was maintained on seedlings of cultivar Williams 82 placed inside the growth chamber at temperatures between 22 and 24°C with a photosynthetically active radiation of 330  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 15 h daily and 60 to 70% relative humidity. The colony was restarted on a fresh batch of seedlings every 3 to 4 wk by transferring aphids from the old seedlings to new ones. These SA were used for all experiments, except one where the SA from University of Illinois was used.

### Greenhouse Screening of Cultivars and Breeding Lines

Thirty-six cultivars and breeding lines, most from Ohio (Supplementary Table 1), were tested in an isolated and pesticide-free greenhouse with heating and cooling facilities. Two resistant checks, PI 567543C and PI 567598B, from Mensah et al. (2005) were included along with the susceptible check Williams 82. The experiment was conducted during September and October of 2005. Plants were grown in 10-cm-deep by 25-cm-wide by 50-cm-long plastic flats with a 8-cm space between plants and a 10-cm space between rows under 15 h light at approximately 24°C days and 9 h dark at 20°C nights. Each experimental unit was a row of three seedlings and five rows were accommodated in a flat. The experiment was arranged in a randomized complete block design (RCBD) with two replicates. At the V1-stage (Fehr and Caviness, 1977), seedlings were infested with 20 to 30 aphids of all developmental stages except the winged aphids by placing an infested leaf section between the petiole of the youngest expanding leaf and the stem. Aphids migrated to the leaves and stems of the seedlings within several hours after putting the infested leaf sections on the seedlings. The aphids were allowed to multiply and move freely among plants in the experiment. The plants were bottom watered to avoid disturbing the aphids. Fourteen days after infestation (DAI), each experimental unit was assigned an aphid score between 1 and 5, where 1 = <25 aphids per plant, 2 = 25 to 100 aphids per plant, 3 = 101 to 200 aphids per plant, 4 = 201 to 400 aphids per plant, and 5 = >400 aphids per plant.

### Greenhouse Screening of MG IV Plant Introductions

One hundred fifty-five MG IV PIs were screened with SA in a greenhouse (Supplementary Table 2). MG-IV PIs from China were selected because only one of the earlier studies (Hill et al., 2004) has investigated a few MG-IV

Table 1. The aphid scores, plant damage scores, and aphid susceptibility indexes from the choice test in field cages.

Line name	Score 14 DAI <sup>†</sup>	Score 28 DAI <sup>‡</sup>	ASI <sup>§</sup>	Comment
PI 243540	1.0	1.0	1.0	Resistant
PI 567301B	1.7	1.0	1.7	Resistant
PI 567324	2.0	1.0	2.0	Resistant
PI 567318	3.0	1.7	5.0	Moderately resistant
PI 567321A	2.3	2.0	4.7	Moderately resistant
PI 567336A	3.7	2.0	7.3	Moderately resistant
PI 567543C	1.3	1.0	1.3	Resistant check (Mensah et al., 2005)
PI 567597C	1.7	1.0	1.7	Resistant check (Mensah et al., 2005)
PI 567598B	2.0	1.0	2.0	Resistant check (Mensah et al., 2005)
Williams 82	4.7	4.5	21.0	Susceptible check cultivar (IL)
Ohio FG5	4.7	4.7	21.7	Susceptible check cultivar (OH)
Wyandot	5.0	4.7	23.5	Susceptible check cultivar (OH)
LSD <sub>0.05</sub>	0.8	0.6	2.8	

<sup>†</sup>Score 1 = <25 aphids per plant, 2 = 25 to 100 aphids per plant, 3 = 101 to 200 aphids per plant, 4 = 201 to 400 aphids per plant, and 5 = >400 aphids per plant. DAI, days after infestation.

<sup>‡</sup>Score 1 = <25 aphids and plant appears normal and healthy, 2 = 25 to 100 aphids per plant and plant appears normal and healthy, 3 = 101 to 300 aphids per plant and plant appears slightly stunted with slight yellowing of older leaves, 4 = 301 to 600 aphids per plant and plant appears moderately stunted with yellowing of older leaves and slight curling of young leaves, and 5 = >600 aphids per plant and plant severely stunted with severely curled and yellow leaves and most of the stem and leaf surfaces covered with sooty mold and cast skins

<sup>§</sup>The aphid susceptibility index (ASI) was calculated by multiplying the aphid score at 14 DAI by the plant damage score at 28 DAI with a possible ASI between 1 and 25.

PIs, while the other two studies (Mensah et al., 2005; Diaz-Montano et al., 2006) did not include any MG-IV PIs. Also, SA has been known to be a pest of soybean in China for a long time (Wu et al., 2004). Seeds were planted in 15 cm deep and 4 cm in diameter plastic cone-tainers and thinned to one seedling per cone-tainer at the unifoliate-stage (Fehr and Caviness, 1977). Each cone-tainer was an experimental unit, and the experiment was arranged in a RCBD with two replicates. At the V1-stage, 20 to 30 aphids of all developmental stages were placed on each plant as described above. Fourteen days after infestation, each plant was assigned an aphid score on a scale of 1 to 5 as described above. On 28 DAI, the plants were evaluated for susceptibility to SA by using a plant damage score (modified from Mensah et al., 2005) of 1 = <25 aphids and plant appears normal and healthy; 2 = 25 to 100 aphids per plant and plant appears normal and healthy; 3 = 101 to 300 aphids per plant and plant appears slightly stunted with slight yellowing of older leaves; 4 = 301 to 600 aphids per plant and plant appears moderately stunted with yellowing of older leaves and slight curling of young leaves; and 5 = >600 aphids per plant and plant appears severely stunted with severely curled and yellow leaves and most of the stem and leaf surfaces are covered with sooty mold and cast skins. The aphid number per plant was included in the plant damage scores because this was particularly helpful in differentiating plants with scores of 1 and 2. In both cases, the plants were fully healthy and

**Table 2. The aphid scores, plant damage scores, and aphid susceptibility indexes from the greenhouse confirmation choice test.**

Line name	Score 14 DAI <sup>†</sup>	Score 28 DAI <sup>‡</sup>	ASI <sup>§</sup>	Comment
PI 243540	1.0	1.0	1.0	Resistant
PI 567301B	2.0	1.0	2.0	Resistant
PI 567324	2.0	1.0	2.0	Resistant
PI 567318	3.2	1.8	5.8	Moderately resistant
PI 567321A	2.5	2.0	5.0	Moderately resistant
PI 567336A	3.7	2.2	8.1	Moderately resistant
PI 567543C	1.4	1.0	1.4	Resistant check (Mensah et al., 2005)
PI 567597C	1.5	1.0	1.5	Resistant check (Mensah et al., 2005)
PI 567598B	2.0	1.5	3.0	Resistant check (Mensah et al., 2005)
Jackson	4.0	4.1	16.4	Resistant check (Hill et al., 2004)
Dowling	4.7	4.6	21.7	Resistant check (Hill et al., 2004)
Williams 82	4.7	4.0	18.8	Susceptible check cultivar (IL)
Ohio FG5	4.7	4.7	21.7	Susceptible check cultivar (OH)
Wyandot	4.8	4.8	23.0	Susceptible check cultivar (OH)
LSD <sub>0.05</sub>	0.7	0.7	2.9	

<sup>†</sup>Score: 1 = <25 aphids per plant, 2 = 25 to 100 aphids per plant, 3 = 101 to 200 aphids per plant, 4 = 201 to 400 aphids per plant, and 5 = >400 aphids per plant. DAI, days after infestation.

<sup>‡</sup>Score: 1 = <25 aphids and plant appears normal and healthy, 2 = 25 to 100 aphids per plant and plant appears normal and healthy, 3 = 101 to 300 aphids per plant and plant appears slightly stunted with slight yellowing of older leaves, 4 = 301 to 600 aphids per plant and plant appears moderately stunted with yellowing of older leaves and slight curling of young leaves, and 5 = >600 aphids per plant and plant severely stunted with severely curled and yellow leaves and most of the stem and leaf surfaces covered with sooty mold and cast skins.

<sup>§</sup>The aphid susceptibility index (ASI) was calculated by multiplying the aphid score at 14 DAI by the plant damage score at 28 DAI with a possible ASI between 1 and 25.

normal looking but a plant with the score of 1 had very few if any aphids (<25) on it while a plant with a score of 2 had up to 100 aphids in some instances. This approach of separating these two types of plants allowed a conservative classification of plants as SA resistant. The plants with relatively higher numbers of aphids were classified as moderately resistant. Also, the aphid numbers at 14 DAI were not always in synchrony with the aphid numbers at 28 DAI. Some moderately resistant plants had the same number of aphids at 14 DAI as the resistant plants, but over the next 2 wk the aphid numbers on these plants increased slowly while the aphid numbers on the resistant plants did not increase at all or declined. The aphid susceptibility index (ASI) (modified from Hill et al., 2004) was calculated by multiplying the aphid score at 14 DAI by the plant damage score at 28 DAI with a possible ASI between 1 and 25.

### Summer Field Evaluation of Selected Lines

Based on the results of the greenhouse screening of MG-IV PIs, three resistant (PI 243540, PI 567301B, and PI 567324) and three of the six moderately resistant (PI 567318, PI 567321A, and PI 567336A) lines along with susceptible and resistant checks were grown in a field near Wooster in 2006. Seeds were planted in hills with 50 by 75 cm spacing among hills. The experiment was arranged in a RCBD with three replicates. Each hill was thinned

to three healthy seedlings at the unifoliate-stage. Each replicate of plants was encaged with nylon mesh coverings supported by 1.8-m high aluminum frames. The edges of the nylon mesh were pegged to the ground to prevent the entry of insect predators or pests into the cage. On June 21 at the V1-stage, each plant was infested by placing an infested leaf section with 20 to 30 aphids on each plant as described earlier. Fourteen DAI, each hill plot was assigned a score between 1 and 5 as described for the greenhouse screening of PIs. Twenty-eight DAI, each hill plot was assigned a damage score between 1 and 5, and an ASI was calculated as described in the greenhouse screening of PIs.

### Confirmation of Aphid Resistance in the Greenhouse Choice Test

During fall 2006, the resistant and moderately resistant PIs from the field-cage study were reevaluated in a second greenhouse choice test. The protocols and environmental conditions for screenings were the same as with the first greenhouse screening of PIs above except for the following modifications. At the V1-stage, each plant was infested by placing 10 adult SA on the youngest partially opened trifoliate leaf of the plant. Dowling and Jackson were included in this test. The experiment was replicated four times in a RCBD. The plants were scored at 14 DAI and 28 DAI using the same scales as described earlier for the greenhouse screening of PIs.

### Confirmation of Aphid Resistance in the Greenhouse No-Choice Test

In February 2007, the six PIs earlier confirmed as resistant or moderately resistant in choice tests, were evaluated in a no-choice (antibiosis resistance) test in the greenhouse under the environmental conditions described earlier. Two plants were grown in a 10-cm plastic pot arranged in a RCBD with four replicates. At the V1-stage, four wingless adults were placed on the upper side of the middle leaflet of the partially opened trifoliate leaf of each plant and the leaflet was immediately encaged with a 5-cm-long by 4-cm-wide by 2-cm-deep polyacrylamide clasp box. The box had two 2-cm-diameter circular holes each netted with aphid proof nylon mesh on opposite sides for cross ventilation. The cage was kept in place by tying it to a stick placed upright next to the plant. Five days after infestation, the cages were opened and the number of live aphids (adults and nymphs) on each infested leaflet was counted.

### Choice Test with the Aphid from Illinois

After greenhouse screening revealed that Dowling and Jackson were susceptible to the SA collected from Wooster, OH, the Ohio aphids were sent to the SA research group

at the University of Illinois at Urbana, IL. Dr. Brian Diers and coworkers conducted a no-choice test using the Ohio aphids on a number of resistant and susceptible soybean lines that they identified earlier by screening with the Illinois aphids. They have confirmed that the Ohio aphids broke the resistance of Dowling and Jackson (B. Diers, personal communication, 2007). We received the Illinois aphid from Dr. Curt Hill in March 2007 and conducted a choice test in a growth room. Three resistant PIs (PI 243540, PI 567301B, and PI 567324) along with check cultivars were grown in 10-cm-wide plastic pots with two seedlings per pot in a growth room at OARDC, Wooster. The experiment had three replicates arranged in a RCBD. The temperature in the growth room was maintained at 24°C with a relative humidity of 60 to 70% and 15 h of light provided from fluorescent bulbs hung 1.2 m above the plants. At the V1-stage, each plant was infested with 10 adult aphids by placing the aphids on the expanding trifoliolate leaves with a paint brush. The aphids were allowed to multiply and move freely among plants in the experiment. Fourteen days after infestation, the number of aphids on each plant was counted.

### Statistical Analyses

The data from each experiment were analyzed using PROC GLM procedure of SAS statistical software V9.1 (SAS Institute, 2002). Means were separated by least significant difference (LSD) at 5% probability level.

## RESULTS AND DISCUSSION

### Greenhouse Screening of Cultivars and Breeding Lines

All cultivars and breeding lines in the experiment except resistant checks were classified as SA susceptible. Any soybean line with an average score of 3.0 or above was considered highly susceptible. The average aphid scores of these lines ranged from 3.3 to 4.1 (Supplementary Table 1). The two resistant lines had a score of 1.3 and the susceptible checks had scores between 3.8 and 4.0.

### Greenhouse Screening of MG IV Plant Introductions

The maximum possible ASI was 25.0. The ASIs for the three resistant checks were <4.0 while the ASI for susceptible cultivars Williams 82 and Ohio FG5 were 19.1 and 21.5, respectively (Supplementary Table 2). The ASI for the 155 PIs ranged from 1.5 to 25.0. Lines with average ASI of <4.0 were classified as SA resistant, lines with average ASI between 4.0 and 8.0 were classified as moderately resistant, and lines with average ASI of >8.0 were considered susceptible. These arbitrary break-points between classes were used, because all resistant checks had ASI <4, and there were two large gaps in ASI (3.6–6.2 and

7.5–10.5) (Supplementary Table 2). Three plant introductions—PI 243540, PI 567301B, and PI 567324—had similar or lower ASI compared to the ASI of the three resistant checks identified by Mensah et al. (2005). Twenty-eight days after infestation, these three PIs and the three resistant checks had less than 50 solitary aphids per plant and showed no typical visual aphid damage symptoms on plants. Six PIs were classified as moderately resistant (Supplementary Table 2). These PIs had 100 to 200 aphids per plant but the number of aphids per plant was much less (20–25%) compared to the number of aphids in the susceptible check cultivars (data not shown). These PIs also showed little or no visible symptoms of aphid damage on plants at 28 DAI. The resistance of these PIs can be termed as partial resistance.

### Summer Field Evaluation of Selected Lines

The results of field evaluation on three resistant and three moderately resistant soybean lines selected based on earlier greenhouse screening were in agreement with the findings from the greenhouse study. Large differences were observed among soybean lines in aphid score at 14 DAI, plant damage score at 28 DAI, and ASI (Table 1). The resistant checks and the three resistant PIs had significantly lower aphid score (<2.0), damage score (1.0), and ASI (<4.0) compared to three moderately resistant PIs and the susceptible checks. The aphid score, damage score, and ASI of the three moderately resistant PIs were intermediate but significantly different from both resistant and susceptible checks. These results indicate that the aphid resistance of the selected lines was equally effective in both greenhouse and field environments in Wooster, OH. While the three PIs designated as resistant had the highest level of resistance against SA, the PIs designated as moderately resistant—PI 567318, PI 567321A, and PI 567336A—showed a level of resistance that clearly separated them from the susceptible check cultivars.

### Confirmation of Aphid Resistance in the Greenhouse Choice Test

The same 12 lines that were evaluated in field cages during the summer 2006 were included in this choice test along with Dowling and Jackson. The results of this experiment confirmed the results from the earlier greenhouse and field screenings. PI 243540, PI 567301B, and PI 567324 were again identified as resistant and PI 567318, PI 567321A, and PI 567336A were identified as moderately resistant (Table 2). Dowling and Jackson were identified as susceptible to the SA (collected from Wooster, OH) used in this experiment. The findings on the susceptibility of Dowling and Jackson were later confirmed at the Univ. of Illinois at Urbana by Dr. Brian Diers and coworkers.

## Confirmation of Aphid Resistance in the Greenhouse No-Choice Test

The no-choice study revealed that SA was unable to multiply and/or survive on PI 243540 when the SA was forced to feed on this PI (Table 3). Thus, PI 243540 exhibited strong antibiosis-type resistance against the SA. The SA was able to survive and multiply on five other resistant and moderately resistant PIs. However, the total number of aphids on each of these lines was significantly less than the number of aphids on the susceptible check cultivar (Table 3). The aphids on these lines appeared smaller than aphids on the susceptible check (data not shown). The resistant PI 567597C with antixenosis-type resistance (Mensah et al., 2005) had a similar number of aphids as these five PIs in the no-choice test. The SA resistance of these five PIs can be characterized as a nonpreference or antixenosis-type. The lesser number of aphids and small sizes of aphids on these PIs compared to those on the susceptible check may also imply a combination antixenosis and antibiosis-type resistances in these PIs.

## Choice Test with the Aphid from Illinois

PI 243540 and PI 567301B were highly resistant against the SA from Illinois with 1.5 and 12 solitary aphids per plant at 14 DAI, respectively. However, PI 567324 showed only a moderate level of resistance with 85 aphids per plant against the SA from Illinois (Table 4). The resistant checks Jackson (Hill et al., 2004) and PI 567543C (Mensah et al., 2005) had 15 and 8 aphids per plant, respectively. The aphids established typical dense colonies on the two susceptible check cultivars (Williams 82 and Wyandot) and had more than 250 aphids per plant.

## Genetic Characterization of the SA Resistant PIs and Their Potential Uses

Genetic characterization of the three resistant PIs is in progress. Segregating populations and backcross lines were developed by crossing these PIs to high-yielding SA-susceptible cultivars from Ohio. The phenotypic segregations of the  $F_1$ ,  $F_2$ , and backcross progeny indicate that SA

resistance in PI 243540 is controlled by a single dominant gene and the resistance in PI 567301B is controlled by two dominant genes (unpublished preliminary results, 2007). These preliminary results will be confirmed by evaluating the segregation of aphid resistance in the corresponding  $F_{2,3}$  families in 2008. Molecular mapping of the SA resistance gene in PI 243540 is also in progress. Using bulk segregant analysis with simple sequence repeat markers, we have located the gene to a region on linkage group F of the consensus soybean genetic map of Song et al. (2004). Thus, the tentative position of this gene is on a different linkage group than linkage group M, on which the *Rag* and *Rag1* genes have been mapped by Li et al. (2007). This is a clear indication that the SA resistant gene in PI 243540 is different from *Rag* and *Rag1*. Two biotypes of SA (one from Ohio and one from Illinois) were recently confirmed and soybean plants with the *Rag* and *Rag1* genes were susceptible to the Ohio biotype of SA (B. Diers, Univ. of Illinois at Urbana, IL, personal communication, 2007). The three SA resistant PIs identified in this study, however, were all resistant to the Ohio biotype of SA. This is another indication that the SA resistant genes in these PIs are different from *Rag* and *Rag1*. The presence of multiple biotypes of SA in the United States presents new challenges to the development of soybean cultivars with resistance to SA. The soybean accessions PI 243540 and PI 567301B with strong resistance to both known biotypes of SA will be useful for developing soybean cultivars with broad based resistance to SA in the United States.

In summary, three MG-IV plant introductions (PI 243540, PI 567301B, and PI 567324) were identified as highly resistant against the soybean aphids collected from soybean fields in Ohio (Ohio biotype). Two of the three plant introductions (PI 243540 and PI 567301B) were also highly resistant to the soybean aphids collected from the fields in Illinois (Illinois biotype). The third plant introduction was moderately resistant against the aphid from Illinois. Three more plant introductions were confirmed to have a moderate level of resistance to the Ohio collection of soybean aphids. These aphid

**Table 3. Number of aphids (all developmental stages) per leaflet 5 d after infestation (DAI) with four adult aphids.**

Line name	No. of aphids 5 DAI	Comment
PI 243540	0.3	Resistant PI
PI 567301B	20.0	Resistant PI
PI 567324	22.0	Resistant PI
PI 567318	20.0	Moderately resistant
PI 567321A	25.0	Moderately resistant
PI 567336A	36.0	Moderately resistant
PI 567597C	15.0	Resistant check (Mensah et al., 2005)
Wyandot	50.0	Susceptible check cultivar (OH)
LSD <sub>0.05</sub>	9.0	

**Table 4. Number of aphids (all developmental stages) per plant 14 d after infestation (DAI) with 10 adult aphids from colony started with SA from Univ. of Illinois at Urbana, IL.**

Line name	No. of aphids 14 DAI	Comment
PI 243540	1.5	Resistant
PI 567301B	12.0	Resistant
PI 567324	85.0	Moderately resistant
Jackson	15.0	Resistant check (Hill et al., 2004)
PI 567543C	8.0	Resistant check (Mensah et al., 2005)
Williams 82	256.0	Susceptible check cultivar (IL)
Wyandot	300.0	Susceptible check cultivar (OH)
LSD <sub>0.05</sub>	39.0	

resistant PIs should be useful in efforts to develop aphid resistant soybean cultivars.

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